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A rapid and highly sensitive portable chemiluminescent immunosensor of carcinoembryonic antigen based on immunomagnetic separation in human serum

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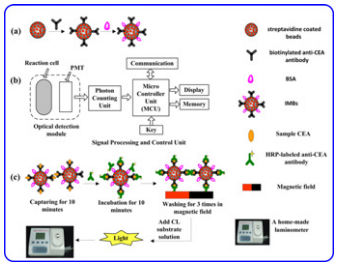
HIGHLIGHTS

- The anti-CEA antibody can bound to the bead with a conjugation rate of 73%.
- IMBs could be stored for 2 months without reduction of biological activity.
- The limit of detection (LOD) of this method was as low as 5.0 pg mL^{-1} ($S/N = 3$).
- The novel immunosensor was highly sensitive with an assay time of <35 min.
- There was a good agreement between our method and ELISA kit.
- A home-made luminometer was used to detect the optical signal.

GRAPHICAL ABSTRACT



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ABSTRACT

To detect a biomarker for lung cancer, carcinoembryonic antigen (CEA), a highly sensitive, selective, rapid and portable immunosensor based on immunomagnetic separation and chemiluminescence immunoassay was introduced. A sandwich scheme assay has been utilized with horseradish peroxidase (HRP) labeled anti-CEA antibody and immunomagnetic beads (IMBs). The presence of target protein CEA caused the formation of the sandwich structures (IMBs-CEA-HRP labeled antibody). IMBs were applied to capture CEA and immobilize CEA through the external magnetic field. The HRP at the surface of the antibody catalytically oxidized the luminescence substrate to generate optical signals which were detected by a portable home-made luminometer and which were directly proportional to the concentration of CEA in the samples. The signals were dependent on CEA concentrations in a linear range from 0 to 50 ng mL^{-1}. The limit of detection (LOD) of this method was as low as 5.0 pg mL^{-1} ($S/N = 3$). The novel immunosensor was highly sensitive with an assay time of <35 min. The intra- and inter-assay coefficients of variation were <10%. The anti-CEA antibody can be bound to the bead efficiently with a conjugation rate of 73%. IMBs could be stored in 4 °C protecting from light for 2 months without obvious reduction of biological activity. Human reference sera mixed with various concentrations of CEA were tested with the proposed

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method and commercial enzyme-linked immunosorbent assay (ELISA) kit, and a good linear relationship was obtained. This proposed technique demonstrated an excellent performance for quantifying CEA and was expected to be used for clinical testing.

1. Introduction

Cancer is one of the major diseases threatening people's health and the incidence is increasing in the world. There will be a predicted global burden of 20.3 million new cancer cases by 2030 compared with an estimated 12.7 million cases in 2008, and a predicted 13.2 million cancer-related deaths worldwide by 2030, up from 7.6 million in 2008 [1]. Tumor markers have been a complementary test in providing both an indication of response to therapy and disease progression or recurrence in patients with cancers. A large number of serum tumor markers have been proposed, such as carcinoembryonic antigen (CEA), carbohydrate antigen 125, alpha-fetoprotein (AFP), neuron specific enolase (NSE) and so on [2–4]. CEA, produced during fetal development, has been the tumor marker most widely investigated. In the mid 1960s, Gold and Freedman isolated CEA from extracts of malignant tissue [5]. It has been reported that CEA is over-expressed in various tumors: colorectal, stomach, pancreas, liver, ovarian, breast, prostate, thyroid, bladder, kidney, and lung [6–8]. Clinically, CEA level in healthy person's body is below 5 ng mL^{-1}.

Immunoassay is an easy and low-cost method for the detection of antigen or its specific antibody in clinical diagnostics. Up to date, various immunoassay methods, such as enzyme-linked immunosorbent assay (ELISA) [9,10], radioimmunoassay (RIA) [11,12], time-resolved fluoroimmunoassay (TRFIA) [13–15], surface-enhanced Raman scattering (SERS)-based immunoassay [16], chemiluminescence enzyme immunoassay (CLEIA) [17,18], have been proposed to meet the clinical analysis. Although the above mentioned methods have low detection limits and are suitable for the determination of CEA in human serum, investigating novel techniques for simple, rapid, sensitive and inexpensive detection of protein markers has attracted many scientists' attention.

In recent years, CLEIA has gained increasing attention [19–22] in different fields because of its high sensitivity, good specificity, wide range of applications, simple equipment and wide linear range [23,24]. Although the CLEIA holds great promise, limitations in the traditional CLEIA method remain. Here, polyclonal antibodies are usually immobilized on a solid substrate in air which will cause several problems. For example, the exposure of proteins to air seriously reduces their biological activity. Also, it requires an extended incubation time because of slow immunoreactions caused by the diffusion-limited kinetics on a solid phase. To solve these problems, we have chosen a combination of immunomagnetic separation (IMS) and CLEIA as a new sensitive process to detect CEA in human serum. Herein, we used microspheres containing superparamagnetic iron oxide nanoparticles (SPION) to form immunomagnetic beads (IMBs) which acted as mobile substrate for isolation of proteins. In this method, the IMBs were employed as capture probes and the HRP-labeled anti-CEA antibody was used as detection probes, then the detection probes and the capture probes formed sandwich structure with sample CEA through antibody–antigen affinity. When the CL substrate was added, detection probes would produce CL signal which was proportional to the concentration of CEA. In order to detect the weak CL signal, a portable luminometer as chemiluminescence transducer was developed. Making IMBs in advance shortened the whole detection time and simplified the operating procedure. To our knowledge, the stability of biofunctionalized magnetic particles and bound antibody determination of IMBs have not been discussed in detail up to now.

2. Experimental

2.1. Reagents

CEA was purchased from Biodesigen (USA). HRP labeled antibody, biotinylated antibody, luminol and H_2O_2 were from Keyue Biotech (Beijing, China). Streptavidine coated magnetic beads (1 μm) were purchased from Dynal Biotech (Invitrogen, Spain). Bovine serum albumin (BSA), phosphate buffered saline were obtained from Sigma (USA). Dilution solution for antigen and antibody was 0.01 mol L^{-1} phosphate buffered saline (PBS, pH 7.4). Washing buffer was PBS with 0.05% Tween-20 (PBST). Stock solution was 0.01 mol L^{-1} phosphate solution with 2% BSA and 0.05% Tween-20. Calibrators were prepared by diluting CEA with PBS solution to target concentrations of 0, 0.005, 0.01, 0.05, 1, 5, 10, 20, 30, 40, 50 ng mL^{-1}, where 0, 1 ng mL^{-1} were assigned to C_0, C_1. All other reagents were of analytical grade. Reagent grade water with a specific resistance of 18.2 M was supplied through a MW-D20 Synthesis (China). The commercial ELISA kit was purchased from Beijing Dongge Biotech. The human reference serum from local hospitals was analyzed without any pretreatment.

2.2. Apparatus

A Shimadzu spectrophotometer (UV1700, Japan) was employed to measure the absorbance of antibody solution and the supernatant at 280 nm so as to calculate the ratio of biotinylated anti-CEA antibody conjugating with magnetic beads. The shaking and incubation procedures at 37 °C were carried out at a thermostatic culture shaker (ZHWY-103B, China). A home-made luminometer was used for chemiluminescence detection. Magnetic separator was provided by Lifengen (Shaanxi, China).

2.3. Procedures

2.3.1. Preparation of IMBs

As illustrated in Scheme 1(a), IMBs were synthesized through the streptavidin–biotin reaction. Firstly, 100 μL of streptavidine coated magnetic beads (10 mg mL^{-1}) were placed into the test tube. And the beads were washed three times with 400 μL of washing solution (PBST) in magnetic field. Then 200 μL of biotinylated anti-CEA antibody (0.2 mg mL^{-1}) were added to the beads and incubated in the shaker for 30 min at 37 °C with 120 rpm min^{-1}. After that, washing was performed with 400 μL of the washing solution five times. Then 1 mL of stock solution was added and shaked for 15 min. Finally, three washing steps were performed and IMBs were resuspended in 1.0 mL of PBS and stored protecting from light at 4 °C for the downstream use.

2.3.2. A portable home-made luminometer

In this study, a portable home-made luminometer was used to convert the chemiluminescence signal into electrical signal. The structure of the luminometer is shown in Scheme 1(b). There were two main units in the system including optical detection module and signal processing and control unit. The optical detection module included a dark reaction cell and a small photomultiplier tube (PMT) module. A PMT with type of H57155-20 (Hamamatsu, Japan) was selected, which was a compact photon counting head device and has stable response for the light with wavelength from